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Cefotaxime and Amoxicillin-Clavulanate Synergism against Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* in a Murine Model of Urinary Tract Infection

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We investigated the efficacies of cefotaxime (CTX) and amoxicillin (AMX)-clavulanate (CLA) (AMC) against extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli in vitro* and in a murine model of urinary tract infection (UTI). MICs, the checkerboard dilution method, and time-kill curves were used to explore the *in vitro* synergism between cefotaxime and amoxicillin-clavulanate against two isogenic *E. coli* strains—CFT073-RR and its transconjugant, CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$—harboring a $bla_{CTX\text{-}M\text{-}15}$ plasmid and a $bla_{OXA\text{-}1}$ plasmid. For *in vivo* experiments, mice were separately infected with each strain and treated with cefotaxime, amoxicillin, and clavulanate, alone or in combination, or imipenem, using therapeutic regimens reproducing time of free-drug concentrations above the MIC (*fT*≥MIC) values close to that obtained in humans. MICs of amoxicillin, cefotaxime, and imipenem were 4/>1,024, 0.125/1,024, and 0.5/0.5 mg/liter, for CFT073-RR and CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$, respectively. The addition of 2 mg/liter of clavulanate (CLA) restored the susceptibility of CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$ to CTX (MICs of the CTX-CLA combination, 0.125 mg/liter). The checkerboard dilution method and time-kill curves confirmed an *in vitro* synergy between amoxicillin-clavulanate and cefotaxime against CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$. *In vivo*, this antibiotic combination was similarly active against both strains and as effective as imipenem. In conclusion, the cefotaxime and amoxicillin-clavulanate combination appear to be an effective, easy, and already available alternative to carbapenems for the treatment of UTI due to CTX-M-producing *E. coli* strains.

The emergence of extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* has been a major public health problem over the last few decades (1). CTX-M-producing *Escherichia coli* strains (especially those producing CTX-M-15) are now common pathogens of urinary tract infections (UTIs) (2). Until recently, carbapenems were uniformly recognized as the treatment of choice of invasive infections due to ESBL-producing *Enterobacteriaceae*. Since the use of carbapenems is clearly recognized as a risk factor for carbapenem resistance (3), there is an urgent need for the evaluation of already available alternatives to carbapenems. Recent data suggest the good efficacy of β-lactam–β-lactamase inhibitor combinations (BL/BLIs) for the treatment of bacteremia due to ESBL-producing *Enterobacteriaceae* (4, 5) when strains remain susceptible to these antibiotics *in vitro*. Nevertheless, the frequent coexistence of the gene $bla_{OXA\text{-}1}$, encoding amoxacillinase, on CTX-M-carrying plasmids, especially CTX-M-15, compromises the efficacy of β-lactamase inhibitors (6). For this reason, the *in vitro* susceptibilities to amoxicillin (AMX)-clavulanate (CLA) (AMC) of ESBL-producing *E. coli* strains are less than 40% in France (7), 30% in the United Kingdom (8), and 16% in India (9). Interestingly, an *in vitro* synergism between extended-spectrum cephalosporins and AMC against CTX-M-15-producing *E. coli* was evidenced (8, 10). Data on the *in vivo* efficacy of such combinations are lacking. The aim of this study was to evaluate the efficacy of the cefotaxime (CTX) plus AMC combination *in vitro* and in a murine model of pyelonephritis due to a CTX-M-15-type ESBL-producing *E. coli* strain.

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MATERIALS AND METHODS

Antibiotics. The antibiotics used in all experiments were provided from manufacturers: cefotaxime (CTX) (Mylan S.A.S., Saint-Priest, France), amoxicillin (AMX) (Panpharma, Luitré, France), potassium clavulanate (CLA) (C9874; Sigma-Aldrich, Saint-Louis, MO), and amoxicillin-clavulanate at a 5:1 ratio (AMC 5:1 [1 g of amoxicillin/200 mg of clavulanate]), which is the formulation used for the human therapeutic in France (Sandoz, Kundl, Austria), and imipenem-cilastatin (IMP) (MSD, Courbevoie, France).

Bacterial strains and media. Experiments were performed with two isogenic strains (11): β-lactam-susceptible *E. coli* CFT073-RR, a clinical uropathogenic strain isolated from the blood of a woman with UTI and already sequenced (12), and its transconjugant, *E. coli* CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$, harboring a plasmid carrying $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, and *aac*(6′)-*Ib*, obtained by conjugation of *E. coli* CFT073-RR with a clinical isolate of community-acquired urinary tract infection in Cambodia (13). All *in vitro* experiments were performed on Mueller-Hinton (MH) broth or agar (Sigma-Aldrich, St-Quentin-Fallavier, France).

***In vitro* experiments. (i) Antibiotic activities.** MICs of AMX, CTX, CLA, IMP, AMX, or CTX plus CLA (with a fixed concentration of 2

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mg/liter of CLA) were determined by the broth macrodilution method in accordance with EUCAST guidelines (14).

Checkerboard synergy testing was performed by the broth macrodilution method. Combinations of CTX and AMC 5:1 were tested at concentrations of 0.06 to 1,024 and 1 to 512 mg/liter, respectively. The fractional inhibitory concentration (FIC) index was calculated by adding the FICs (MIC of drug A in combination with drug B/MIC of drug A alone) of CTX and AMC 5:1. Synergy was defined as a FIC index of ≤0.5 (15).

For time-kill curves, exponentially growing *E. coli* cells were diluted in glass tubes containing 10 ml of broth to obtain 10^5 CFU/ml (standard inoculum) or 10^7 CFU/ml (high inoculum) and incubated with CTX (1 or 4 mg/liter) and/or AMC (4 or 8 mg/liter). Viable counts were enumerated by plating 100 μl of appropriate culture dilutions onto MH agar plates after 0, 1, 3, 6, and 24 h of incubation at 37°C and expressed in $\log_{10}$ CFU per milliliter. The lower limit of detection was 1 $\log_{10}$ CFU/ml. A bactericidal effect was defined as a decrease of at least 3 $\log_{10}$ in CFU counts compared to the initial inoculum. A synergistic effect was defined as a ≥2-$\log_{10}$ decrease in CFU counts between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination had to be ≥2 $\log_{10}$ CFU/ml below the starting inoculum. Antibiotic carryover did not interfere with bacterial counts at the antibiotic concentrations used (16). All *in vitro* experiments were repeated at least three times.

(ii) Drug pharmacokinetics and antibiotic assays. Pharmacokinetic (PK) studies were performed on uninfected mice in order to determine the therapeutic regimen of CTX and AMC that best reproduced the same percentage of time of free drug concentrations above the MIC (*fT*≥MIC) and the same plasmatic peak concentrations of antibiotics as those obtained in humans with standard regimens according to the current practice: i.e., CTX, 1 g every 8 h (q8h), AMC, 1 g q8h; and IMP, 1 g q8h (17–23).

Blood samples of at least 800 μl were obtained by intracardiac puncture from 4 anesthetized mice 15, 30, 60, 120, 240, and 360 min after a single subcutaneous infection of CTX (100 mg/kg) or AMC 5:1 (100 mg/kg). For IMP (100 mg/kg), data from a previous study from our laboratory were used (11). After centrifugation, concentrations were determined by high-performance liquid chromatography with UV detection at 237 nm. The limits of quantification were 0.5 mg/liter for CTX, 1 mg/liter for AMX, and 0.5 mg/liter for CLA. The intraday and extraday precisions were 4 to 7.7%, 3.5 to 5.6%, and 4 to 9.1% for CTX, AMX, and CLA, respectively.

Free drug concentrations were determined after ultrafiltration by centrifugation in a Centrifree YM-30 (Millipore SAS, Molsheim, France) at 2,000 × *g* and 25°C for 10 min. The *fT*≥MIC values in mice were obtained from observed data by linear regression.

(iii) Mouse model of pyelonephritis. Animal experiments were approved by the local ethics committee (Departmental Direction of Veterinary Services, Paris, France, agreement no. 75-861). The ascending, unobstructed UTI mouse model was used as previously described (11, 24). Eight-week-old immunocompetent CBA female mice (weight, 20 to 23 g) were used. Inocula were obtained by overnight incubation of strains in broth at 37°C, washing of cells by centrifugation at 4,000 × *g* for 15 min, and resuspension in 1 ml saline to a final inoculum of 10^{10} CFU/ml. After general anesthesia of mice, pyelonephritis was induced by injecting 50 μl (5.10^8 CFU) of the inoculum into the bladder through a urethral catheter. In this model, strains were able to induce stable pyelonephritis in mice with maximal infection between days 5 and 10 without plasmid loss at day 10 (11).

For each therapeutic regimen, 25 mice were infected. Five days after inoculation, 5 mice were sacrificed (start-of-treatment controls), 5 were left untreated (end-of-treatment controls), and 15 were treated over 24 h by subcutaneous injections of CTX, AMC 5:1, CTX plus AMC 5:1, or IMP. End-of-treatment controls and treated mice were sacrificed 24 h after the last dose of antibiotic to avoid carryover (at day 7). Kidneys were aseptically taken out, weighed, and then homogenized in 1 ml of saline solution. After 48 and 72 h of incubation at 37°C, CFU counts were enumerated on MH agar plates with and without 1 mg/liter of CTX and expressed as $\log_{10}$ CFU per gram of kidney. When no growth was observed on agar plates after 72 h, kidneys were considered sterile, and the value was considered the limit of quantification, defined as the $\log_{10}$ CFU per gram of kidney corresponding to the growth of 1 CFU and to the weight of kidneys in the individual mouse.

Statistical analysis. Results are expressed as the median followed by the range (minimum to maximum) in parentheses or brackets for continuous variables. Mean $\log_{10}$ CFU gram in kidneys were compared for the different groups by the Mann-Whitney U test. Proportions of sterile organs were compared by Fisher's exact test. Comparative analysis of the maximum growth rate (MGR) was performed by the Wilcoxon test. All statistical analyses were performed using BiostaTGV (R 3.0.0 software) (http://marne.u707.jussieu.fr/biostatgv/). A *P* value of <0.05 was considered significant.

TABLE 1 MICs of antibiotics against *E. coli* strains determined by the broth macrodilution method

E. coli strain	MIC (mg/liter)[a]					
	AMX	CLA	AMX-CLA[b]	CTX	CTX-CLA[b]	IMP
CFT073-RR	4	32	4	0.125	0.125	0.5
CFT073-RR Tc *bla*$_{CTX-M-15}$	>1,024	32	256	1,024	0.125	0.5

[a] AMX, amoxicillin; CLA, potassium clavulanate; CTX, cefotaxime; IMP, imipenem.
[b] Fixed clavulanate concentration of 2 mg/liter according to EUCAST recommendations (13).

RESULTS

MICs and checkerboard dilution method. The MICs of each antibiotic tested against isogenic strains are presented in Table 1. High levels of resistance to CTX and AMX plus CLA were observed for *E. coli* CFT073-RR Tc *bla*$_{CTX-M-15}$; addition of 2 mg/liter of CLA did not restore the susceptibility to AMX but fully restored the susceptibility to CTX. The addition of 2 mg/liter of CLA had no effect on MICs of AMX and CTX against susceptible CFT073-RR. Results of the checkerboard dilution method between CTX and AMC 5:1 against CFT073-RR Tc *bla*$_{CTX-M-15}$ at two different inocula are presented in Fig. 1. For this strain, a synergism between CTX and AMC 5:1 was observed. An AMC 5:1 concentration of 4 mg/liter at the standard inoculum and 8 mg/liter at the high inoculum was necessary to obtain a CTX MIC of 1 mg/liter (FIC of 0.06 for both). No synergy was observed between AMC 5:1 and CTX against the susceptible strain (lower FIC of 0.75) (data not shown).

Time-kill curves. Figure 2 shows the activity of the CTX and AMC 5:1 used alone or in combination against the CFT073-RR Tc *bla*$_{CTX-M-15}$ strain. At a standard inoculum, CTX at 1 mg/liter or AMC 5:1 at 4 mg/liter did not impair bacterial growth. In contrast, the combination of CTX (1 mg/liter) and AMC 5:1 (4 mg/liter) was synergistic and bactericidal at 24 h (Fig. 2A). With a higher initial inoculum, these combinations led to an initial decrease in bacterial counts, but regrowth was observed at 24 h. A synergistic and bactericidal effect was restored when the CTX concentration was increased from 1 to 4 mg/liter or the AMC 5:1 concentration was increased from 4 to 8 mg/liter (Fig. 2B).

Pharmacokinetics and pharmacodynamic parameters. The pharmacokinetic (PK) and pharmacodynamic (PD) parameters measured by peak value, protein binding, and *fT*≥MIC in mice are shown in Table 2. For CTX, the optimal therapeutic dosing regimen of 100 mg/kg q2h led to an *fT*≥MIC of 100%

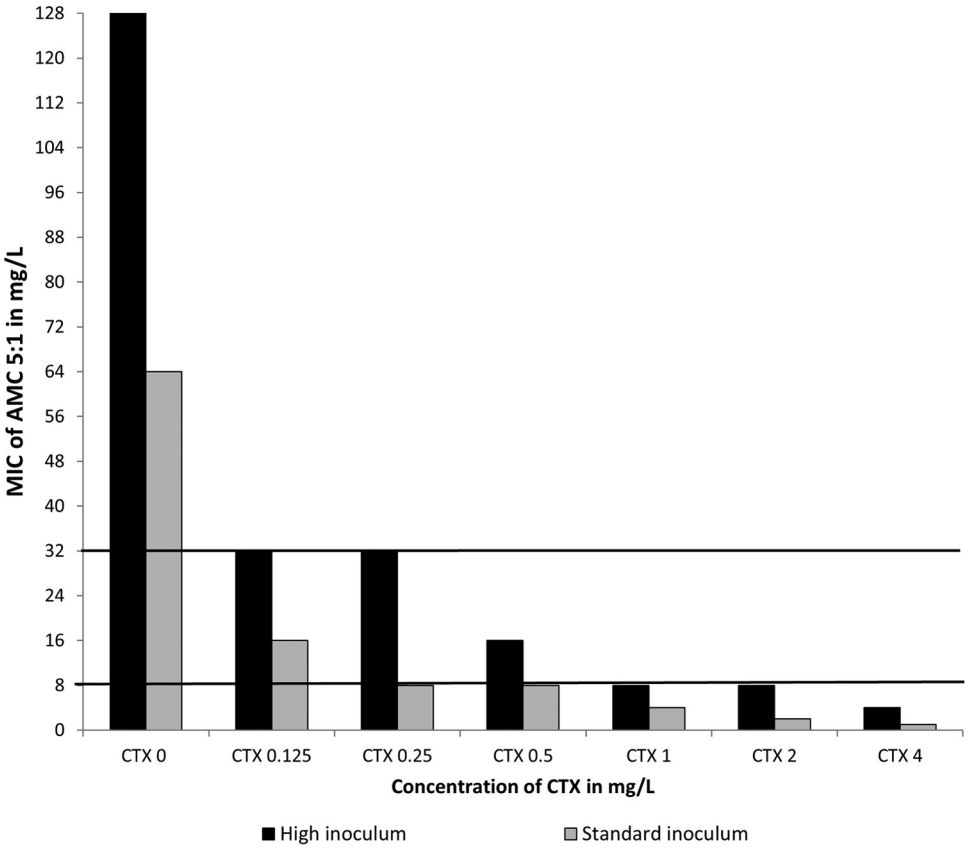

FIG 1 MICs of AMC 5:1 in combination with various concentrations of CTX against CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$ at two different inocula, assessed by the checkerboard broth dilution method. AMC 5:1, amoxicillin-clavulanate with a 5:1 ratio; CTX, cefotaxime; standard inoculum, 10^5 CFU/ml; high inoculum, 10^7 CFU/ml. The upper and lower limits (black lines) correspond to EUCAST susceptibility breakpoints for AMC for urinary tract infections and systemic infections, respectively.

(close to that obtained in humans with 1 g 3 times/day) (25, 26) against CFT073-RR. For AMC 5:1, an optimal dosing regimen of 100 mg/kg q4h led to a peak of 94.5 mg/liter and an $fT \geq$MIC of 42% (close to that obtained in humans with 1 g/200 mg 3 times/day) (19, 21) against CFT073-RR. Both of these optimal therapeutic regimens led to an $fT \geq$MIC of 0% against CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$. To study the effect of the combination, both antibiotics were injected together at 100 mg/kg every 4 h.

Mouse model of pyelonephritis. Both strains were able to induce stable pyelonephritis in mice without any significant difference in bacterial counts in kidneys between the start-of-treatment (median, 4.89 $\log_{10}$ CFU/ml [range, 1.61 to 6.51] and 4.74 $\log_{10}$ CFU/ml [range, 3.44 to 6.35] for CFT073-RR and for CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$, respectively) and the end-of-treatment control groups (median, 5.03 $\log_{10}$ CFU/ml [range, 1.55 to 6.21] and 5.02 $\log_{10}$ CFU/ml [range, 1.44 to 7.19] for CFT073-RR and CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$, respectively).

In mice infected with CFT073-RR, all therapeutic regimens tested produced significant reductions in viable bacterial counts compared to end-of-treatment control mice (Table 3). The CTX and AMC 5:1 combination was more active than AMC 5:1 or CTX used alone with a similar therapeutic regimen (every 4 h), while no difference was observed with CTX at 100 mg/kg q2h or IMP ($P = 0.32$ and $P = 0.11$, respectively). There was no statistical difference in the proportion of sterile kidney whatever the antibiotic regimen used (Table 3). In mice infected with CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$, the CTX and AMC 5:1 combination was as active as IMP and significantly more active than CTX (100 mg/kg q4h and 100 mg/kg q2h) or AMC 5:1 alone in terms of both decrease in bacterial counts and kidney sterilization (Table 3). Furthermore, the decrease in bacterial counts observed with the CTX and AMC 5:1 combination against CFT073-RR Tc $bla_{CTX\text{-}M\text{-}15}$ was not different from that observed with CTX 100 mg/kg q2h ($P = 0.610$) or the CTX and AMC 5:1 combination ($P = 0.40$) against CFT073-RR (Table 3).

DISCUSSION

In vitro synergy between third-generation cephalosporins (3GC) and β-lactamase inhibitors against ESBL-producing *Enterobacteriaceae* has already been reported (8, 10), but reports on the efficacy of such combinations *in vivo* are scarce. Only a few countries possess 3GC and β-lactamase inhibitor combinations available in a single formulation, discouraging physicians from using such combinations.

We chose to evaluate the *in vivo* efficacy of 3CG and β-lactamase inhibitors using two low-cost and available antibiotics: CTX and AMC 5:1. There is only one publication related to successful treatment with cefixime plus AMC combination in a relay with imipenem in two children with pyelonephritis (27).

To our knowledge, this report brings the first demonstration of

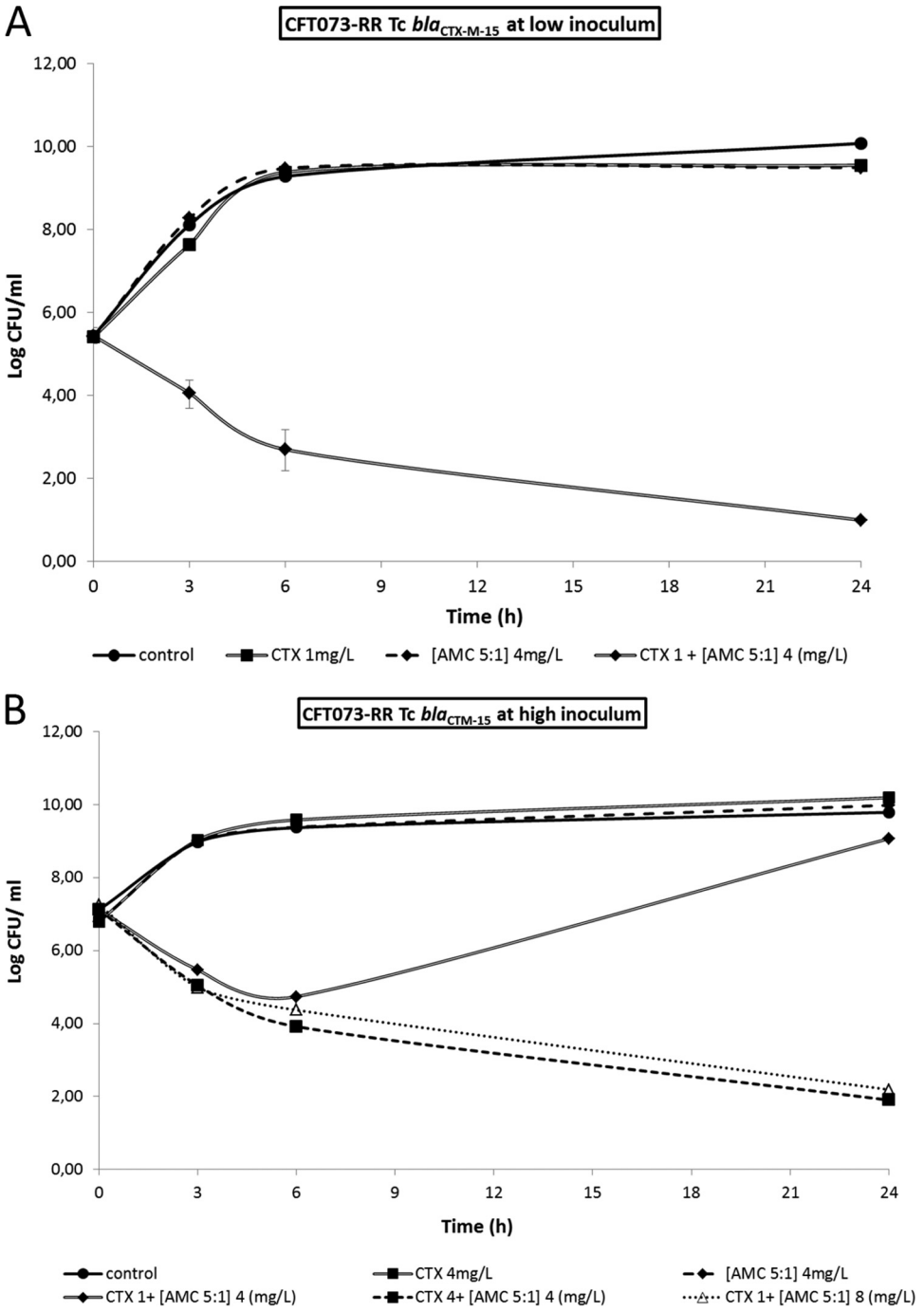

FIG 2 Time-kill curves against CFT073 RR Tc $bla_{CTX\text{-}M\text{-}15}$ at various inocula. Shown are the bactericidal activities of CTX, AMC 5:1, and their combination against CFT073 RR Tc $bla_{CTX\text{-}M\text{-}15}$ at standard (10^5 CFU/ml) (A) and high inocula (10^7 CFU/ml) (B). AMC 5:1, amoxicillin-clavulanate at a 5:1 ratio; CTX, cefotaxime.

the *in vivo* efficacy of such a combination against CTX-M- and OXA-1-producing *E. coli*. The combination was as effective as imipenem; in addition, the activity of the combination against the ESBL-producing strain was comparable to that of an optimal CTX regimen against the susceptible parental strain.

Therapeutic regimens of cefotaxime or amoxicillin-clavulanate at 100 mg/kg q4h permitted us to achieve an $fT{\geq}$MIC of over 40% against the susceptible strain and can be considered effective according to reports by Craig et al. (28). A therapeutic regimen of cefotaxime at 100 mg/kg q2h in mice provided an $fT{\geq}$MIC of around 100%, close to that obtained in humans with 1 g 3 times per day when the MIC of infecting strain is 0.125 mg/liter (25). Nevertheless, we preferred to use a relatively suboptimal regimen of the combination in order to better assess the effect of the synergy by avoiding a cumulative dose effect and pharmacokinetic interferences.

TABLE 2 Antibiotic dosing regimen and corresponding pharmacokinetic/pharmacodynamic parameters against susceptible and resistant *E. coli* strains

Antibiotic[a]	Dosing regimen in mice	Result for parameter[b]: C_{max} (mg/liter)	Protein binding (%)	*fT*>MIC, % (MIC, mg/liter) *E. coli* CFT073-RR	*fT*>MIC, % (MIC, mg/liter) *E. coli* CFT073-RR $bla_{CTX-M-15}$
AMC 5:1	100 mg/kg q4h	94.5	20[c]	42 (4)	0 (256)
CTX	100 mg/kg q4h	118	12	50 (0.125)	0 (1,024)
	100 mg/kg q2h	118	12	100 (0.125)	0 (1,024)
IMP	100 mg/kg q2h	91	34	87 (0.5)	87 (0.5)

[a] AMC 5:1, amoxicillin-clavulanate at a 5:1 ratio; CTX, cefotaxime; IMP, imipenem.
[b] C_{max}, peak value of total concentration; *fT*>MIC, percentage of time of the dosing interval during which free-drug concentrations remained above the MIC of the corresponding strain.
[c] See reference 18.

In vitro, the MIC of CTX against CTX-M-producing *E. coli* decreased more than 8,000-fold after addition of 2 mg/liter of CLA. This dramatic *in vitro* synergy was also observed when using the checkerboard macrodilution method and by time-kill curves (8). A mild *in vitro* inoculum effect was observed, but bacteriostasis and the bactericidy of the combination were restored by increasing 2- or 4-fold the AMC 5:1 concentration. Our results are in accordance with previous reports showing that CLA is much less affected by the inoculum effect than other β-lactamase inhibitors (29). However, due to the relatively low bacterial inoculum in kidneys in our model, we cannot exclude a potential inoculum effect in other foci of infection where the inoculum is higher.

Other limitations related to our animal model could be underlined as follows. (i) The ESBL-producing strain was not a clinical strain but a transconjugant of uropathogenic *E. coli* CFT073. This limitation does not allow us to extrapolate our results to ESBL-producing clinical isolates. However, since both strains had the same MGR and infectivity profile in our murine model, the same results can be expected from clinical isolates. (ii) Only one ESBL type was analyzed in our *in vivo* model; for ethical and technical reasons, we could not evaluate the *in vivo* activity of the combination against a large panel of strains. However, on the basis of *in vitro* literature data (8, 10), and since our strain was highly resistant to 3GCs and AMC, similar *in vivo* results may be expected from other ESBL-producing *E. coli* strains.

CLA may induce high-level expression of chromosomal or acquired AmpC β-lactamases. Although AmpC enzymes are not the most important problem in third-generation-cephalosporin resistance (3GC-R) in France and Belgium compared to ESBL enzymes for *E. coli* (5.7% versus 94%) or *Klebsiella pneumoniae* (7.3% or 92.8%) (30), chromosomal AmpC overproduction accounts for 58% of 3GC-R in *Enterobacter* spp., *Morganella* spp., *Serratia* spp., *Providencia* spp., and *Citrobacter freundii* (30). For these species, combination of CLA with cefepime may offer an advantage. This combination already showed a good *in vitro* activity (8, 31) and deserves *in vivo* evaluation.

Among β-lactam alternatives to carbapenems for the treatment of severe infections due to ESBL and OXA-1 producers, cephamycins (32) and temocillin are the most promising antibiotics. However, treatment of ESBL-producing *K. pneumoniae* by cefoxitin was associated with emergence of porin-deficient resistant mutants responsible for therapeutic failure and human death (33). Looking at *E. coli*, MICs of temocillin and cefoxitin against ESBL-producing *E. coli* are higher than those of the CTX-CLA combination. Previous reports from our team found a MIC of temocillin against CFT073-RR Tc $bla_{CTX-M-15}$ between 8 and 32 mg/liter (according to the method used—agar or macrodilution, respectively) and a MIC of cefoxitin of 4 mg/liter (agar dilution) versus a MIC of 0.125 mg/liter for CTX plus CLA (macrodilution) (11, 23). This better *in vitro* efficacy, associated with our *in vivo* data, suggests that CTX and AMC 5:1 combination may play a leading role among possible alternatives to carbapenems. The CTX and AMC 5:1 combination has the advantage of being widely

TABLE 3 Effect of antibiotics on viable organisms in kidneys of mice infected with *E. coli* strains CFT073-RR and CFT073-RR Tc $bla_{CTX-M-15}$

Antibiotic dosing regimen[a]	Result for *E. coli* strain: CFT073-RR Median $\log_{10}$ CFU/g kidney (range)[b]	CFT073-RR No. sterile/total	CFT073-RR Tc $bla_{CTX-M-15}$ Median $\log_{10}$ CFU/g kidney (range)[b]	CFT073-RR Tc $bla_{CTX-M-15}$ No. sterile/total
End-of-treatment control	5.03 (1.55–6.21)	1/20	5.02 (1.44–7.19)	0/22
AMC 5:1, 100 mg/kg q4h	1.95 (1.61–5.30)[c]	5/13[c]	3.68 (1.52–6.59)	2/17
CTX				
100 mg/kg q4h	1.94 (1.56–3.92)[c]	5/13[c]	4.39 (2.27–5.98)	0/13
100 mg/kg q2h	1.86 (1.52–3.30)[c]	8/15[c]	4.12 (1.61–6.74)	3/15
CTX-AMC 5:1, 100 mg/kg q4h	1.60 (1.50–2.91)[c,d,e]	9/13[c]	1.60 (1.52–5.21)[c,d,e,f]	10/17[c,d,e,f]
IMP, 100 mg/kg q2h	1.87 (1.53–5.25)[c]	6/14[c]	1.60 (1.52–4.65)[c,d,e,f]	12/15[c,d,e,f]

[a] AMC 5:1, amoxicillin-clavulanate at a 5:1 ratio; CTX, cefotaxime; IMP, imipenem.
[b] The results are expressed as median $\log_{10}$ CFU per gram of kidney (range) for mice treated with the corresponding antibiotic regimen.
[c] $P < 0.05$ compared with the end-of-treatment controls for the corresponding strain.
[d] $P < 0.05$ compared with the AMC 5:1 100-mg/kg q4h group for the corresponding strain.
[e] $P < 0.05$ compared with the CTX 100-mg/kg q4h group for the corresponding strain.
[f] $P < 0.05$ compared with the CTX 100-mg/kg q2h group for the corresponding strain.

used, with a well-known tolerance profile and a low cost. Other 3GC and AMC 5:1 combinations, including cefixime or cefpodoxime, are worth being evaluated *in vivo*, since they offer the advantage of oral formulations. However, the ecological impact of such broad-spectrum combinations needs to be evaluated.

In conclusion, the CTX and AMC 5:1 combination appear to be an effective, easy, and already available alternative to carbapenems for treatment of pyelonephritis due to CTX-M-producing *E. coli*. Additional studies are necessary to evaluate its efficacy in other foci of infection with a higher bacterial inoculum.

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